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Studies on Lymphocystis Disease in the Orange Filefish, *Ceratacanthus schoepfii* (Walbaum), from Sandy Hook Bay, N. J.

Ross F. Nigrelli
New York Aquarium
&
G. M. Smith
Department of Anatomy, Yale School of Medicine, and New York Aquarium.

(Plates I-VIII).

Introduction.

Lymphocystis disease is characterized by the development of small, irregular, solitary or confluent nodules of grayish-white color on the skin and fins of marine and fresh-water fishes. It was first reported as multiple tumors from the European flounders, *Pleuronectes flesus* and *P. platessa,* by Lowe (1874), McIntosh (1884, 1885) and Sandeman (1892). Microscopically, the disease appears as large cells, each surrounded by a thick membrane. The cells contain large nuclei, nucleoli, and cytoplasmic inclusions, the latter appearing either as discrete bodies or in the form of one or more networks. Woodcock (1904) believed these cells to be sporozoa, which he named *Lymphocystis johnstonei.* Since then various discussions have appeared in the literature concerning the true identity of the disease. Johnstone (1905) agreed with Woodcock as to its protozoan nature. A similar interpretation was given by Awerinzew (1907, 1909, 1911). He believed that the enlarged cells were stages in the life-history of a myxosporidian, which he called *Henneguya johnstonei* (Woodcock), disagreeing with Woodcock as to its taxonomic position. According to Awerinzew (1911), the youngest cells are minute in size and have a single nucleus. The cells grow rapidly, and in the process, chromatin passes out from the nucleus to form a chromidial ring in the cytoplasm. From this ring secondary nuclei are formed, around which bits of cytoplasm are cut off to form small cells referred to as "secondary ameboids." These are compared to sporonts of *Glugea.* Further, within the "secondary ameboids," spores are formed, the detailed structures of which were not clearly definable. Minchin (1912), reporting on this subject and on Woodcock's paper, regarded them as doubtful protozoa. Doflein (1928) included *Lymphocystis johnstonei* as one of the neosporidians although he recognized the controversy involved.

Mavor (1918) was the first to report the disease in America, having discovered the condition on the pike-perch, *Stizostedion vitreum.* He also thought that the cells were neosporidians, but considered them sufficiently different from the European *Lymphocystis* to propose the name *Lymphocystis vitrel.*

Zschieche (1910) was first to report the disease in the fresh-water paradise fish, *Macropodus*. However, he failed to recognize the true nature of this condition because he believed that the large cells observed were some form of fish ova.

Other investigators, however, are of the opinion that these cells are not parasites, but consider them as greatly hypertrophied host cells. This view was first formulated by Weissenberg (1914, 1920, 1921 b, c) who produced histological and cytological evidence that these "giant" cells were hypertrophied connective tissue cells. This evidence was deduced partly from periodic examination of experimentally produced tumors, especially those obtained in *Acerina cernua* and *Pleuronectes flesus*.

Similar interpretation was given to the disease found in *Sargus annularis* by Joseph (1917, 1918); for the European flounder, *Pleuronectes flesus*, by Claussen (1917), Plehn (1924), Johnstone (1926) and Raab (1935); for *P. platessa* and the paradise fish, *Macropodus*, by Schäperclaus (1935); for the East Indian coral fishes, *Premnas biaculeatus* and *Amphiprion percula*, by Benisch (1937). Lymphocystiosis was first demonstrated for the American Atlantic seaboard in the West Indian angelfish, *Angelichthys isabelita*, by Smith & Nigrelli (1937); for the hogfish, *Lachnolaimus maximus*, by Weissenberg, Nigrelli & Smith (1937); for the orange filefish, *Aleutera schoepfii*, by Weissenberg (1938); and for the common killifish, *Fundulus heteroclitus*, by Weissenberg (1939).

It is of interest to mention here that the disease described by Gilruth & Bull (1912) as *Lymphocystis macropodus* must not be confused with the condition reported by the above investigators. According to Gilruth & Bull, *L. macropodus* is a sarcosporidian-like parasite found in the intestinal mucosa of the kangaroo (*Macropus* sp.). Wenyon (1926), however, refers this parasite to the genus *Globidium*.

Nature of Cytoplasmic Inclusions in Lymphocystis Cells.

Although Awerinzew (1911) considered the lymphocystis cells as protozoan, he nevertheless recognized the fact that the inclusion bodies found in the cytoplasm were made up of chromatin material given off by the nucleus. This chromatin material has been referred to as "chromidia," and he further believed that this was a normal nuclear behavior similar to that recognized among certain protozoa preparatory to "spore" formation.

Weissenberg (1914-1939) considers the inclusions as basochromatin and the tremendously developed network found in older hypertrophic cells as the result of growth of a granule of basochromatin which has the appearance of a Guarnieri-like body. In his later papers he reports that the cell hypertrophy is due to an intracellular virus. Joseph (1918) considered the reticulation to be modified and much hypertrophied centrophormium, a point disputed by Weissenberg (1921a). Jirovec (1932), employing a modified Feulgen's technique, concluded that the inclusions in lymphocystis cells were nuclear in origin, giving a positive reaction for thymonucleic acid. Raab (1935) concluded that the cell hypertrophy in lymphocystis was the result of an intracellular protozoan parasite (microsporodian) which he tentatively placed in the genus *Glugea*. Such intracellular organisms also have been known to cause tremendous cell hypertrophy. For example, *Glugea* (*Nosema*) *anomala*, parasitic in wandering connective tissue cells of the stickleback, enlarges from a cell 8 microns in diameter and containing a single organism to one 4,000 microns in diameter and containing thousands of spores (*vide* Weissenberg, 1921d, 1937). Hyde (1937) in his laboratory outline of filterable viruses considers lymphocystis a virus disease, following Weissenberg's opinion.

LYMPHOCYSTIS IN ORANGE FILEFISH FROM SANDY HOOK BAY, N. J.

Four orange filefish caught in the waters of Sandy Hook Bay, New Jersey, in August, 1938, were found heavily infected with lymphocystis; two taken in September of that year showed no external signs of the disease. Although the size and form of the external tumors varied considerably, the gross appearance was typical. These gross lesions are pictured in the photographs in Pl. I, Figs. 1 and 2. The material was freshly fixed in 10% neutral formalin, decalcified and stained with hematoxylin-eosin, Giemsa's or Mallory's triple stain. Some of the material was used in attempting experimental transmission.

Histological observations on the orange filefish material at our disposal indicate that the disease is not limited to a cutaneous manifestation, but that internal organs are involved as well. Material recently studied showed the presence of typical hypertrophic cells in the gastro-intestinal tract, spleen and ovary. These visceral lesions together with the cutaneous ones, will be discussed later in this paper.

Microscopically, the fully developed lymphocystis cells are seen in Pl. I, Fig. 3. In Pl. II, Fig. 4, the tumor has formed a small pedunculated mass attached to the fin by a fibrous stalk. The small tumor is composed entirely of fully developed lymphocystis cells, separated by a meshwork of dense hyalin material usually referred to as the hyalin membrane of the cell. Where the cells form isolated units in adjacent tissues the membrane closely surrounds the cytoplasm of each cell (Pls. V, VI, VII), but in the tumor mass the cell membrane material appears to be a confluent and connecting network (Pl. I, Fig. 3; Pls. II, III, IV). Outstanding as well is the fact that this membrane-like material is laid down early in the development of the hypertrophic mesenchymal cell, the latter eventually growing to typically gigantic size. The cell membrane material appears to be continuous at times with the more delicate reticulum of adjacent normal areas.

Whenever the lymphocystis cells are highly developed and closely packed together to form a tumor, ordinary connective tissue stroma of the tumor proper is very scanty and the blood supply is not a rich one. Epithelium overlying small nodules may either remain unchanged or be somewhat thickened.

The present material has afforded an opportunity to study collections or nests of growing lymphocystis cells, and certain progressive stages from the young fibroblastic phase up to the extremely hypertrophied adult cells. These observations on progressive stages of cell hypertrophy tend to confirm Weissenberg's (1914-1938) interpretation that the disease is primarily a cell hypertrophy affecting host connective tissue cells. It is further demonstrated in this study that the hypertrophic cells can be recognized at a very early stage (cells measuring 8.3 microns or 6 x 21 microns). At this stage, the hyalin membrane already has been developed; the cytoplasmic material is somewhat increased in amount over the normal; and the nucleus decidedly enlarged and deeply pycnotic. The shape of the young lymphocystis cells may be round, oval or fusiform. Certain early stages (9 x 24 microns) show cells in what resembles a binucleate condition (Pls. III, IV). In other cells (20 x 50 microns) the nuclear material appears in two forms (Pl. III, Figs. 6, 7): (a) as a deeply pycnotic primary nucleus which later hypertrophies to remain as the nucleus of the enlarged cell, and (b) a secondary nuclear mass, variable in size, which becomes vacuolated and reticulated. The latter mass of basophilic material represents probably the forerunner of the system of inclusion bodies which eventually become distributed in the cytoplasm of the enlarged older cell (Pl. IV, Fig. 9; Pl. V, Fig. 10). In these fully developed cells (see also Pl. VII, Fig. 14; and Pl. VIII) the chromatin forming the cell inclusion bodies is frequently dispersed in the region of the periphery of the cell and nucleus, either as a reticulum or as isolated chromatin granules. Such an extremely developed cell as

shown in Pl. V, Fig. 10, may measure 456 x 608 microns, the hypertrophied nucleus 54 microns, and the hypertrophied nucleolus 10 microns.

The ultimate fate of the lymphocystis cells is as yet not clearly understood, and has not been the subject of previous reports. In some of the sections of the orange filefish tumors composing our material, numerous cells appeared in various stages of degeneration. Since the material was immediately fixed, post-mortem autolytic changes can be probably excluded. In several preparations made from fishes in various stages of recovery, a healing phase of the lesion was demonstrated. It was noted here that the cell membranes were collapsed, lying in a thick fibrous scar tissue exhibiting no recognizable cell content except for small amounts of degenerated material. These degenerative changes, in conjunction with the collapse of cell membranes, suggest that lymphocystis cells have either died *in situ* or that the membranes have ruptured permitting evacuation of possible viral contents into adjacent tissue or into the surrounding water, depending on the immediate location of the cells. The collapsed membranes assume a bizzare appearance as seen in Pl. V, Fig. 11. The original shape is greatly distorted. The walls become approximated and infolded. The thickness of the membrane may be less than usual or greatly increased. Plate VI, Fig. 13, shows a degenerating lymphocystis cell in the spleen. A distinct break in the continuity of the membrane of the cell can be seen, resulting in an invasion of splenic tissue within the cyst cavity. In some of the collapsed cysts in cutaneous regions, loosely arranged connective tissue was noted within the cavity of the degenerated cells. Collections of lymphoid cells in the firm fibrous tissue surrounding collapsed cell membranes are not uncommon.

That lymphocystis disease is not limited to cutaneous lesions has been indicated by Woodcock (1904) and Awerinzew (1909) who reported having found these enlarged cells in the mesentery, intestine and ovary. However, illustrations do not accompany these observations. The study of the lymphocystis disease in orange filefish from Sandy Hook Bay indicates clearly that the lymphocystis disease cannot be regarded alone as a cutaneous lesion in this fish. In one of the specimens, the spleen showed numerous lymphocystis cells fully developed, usually lying in the substance of the splenic pulp. Plate VI, Fig. 12, shows a lymphocystis cell in the spleen in good condition, stained with hematoxylin-eosin, and measuring 364.8 x 480.4 microns. The splenic tissue surrounding the hypertrophied cell has a somewhat concentric arrangement. The infected spleen in this fish was greatly enlarged and on gross section the isolated lymphocystis cells could be readily distinguished by the naked eye or with a small hand lens.

A few lymphocystis cells were also encountered in the gastro-intestinal tract, as shown in Pl. VII and Pl. VIII, Fig. 16. One hypertrophied cell was found to occupy a position directly below the surface epithelium (Pl. VII, Fig. 14), being separated at the surface by a thin layer of flattened host cells (gastric epithelium). The hypertrophied nucleus in this cell showed fairly dense granular substance containing minute basophilic material. The hypertrophied nucleolus had a thick margin and contained closely packed spherical globules with lighter staining centers. In other areas cells were found lying deeper in the connective tissue fold (Pl. VII, Fig. 15). Here the cell lies almost in the middle of the fold, in close relation doubtless to the blood and lymphatic vessels of the intestinal wall. In Pl. VIII, Fig. 16 the lymphocystis cell lies below the submucosa and within the fold. In the latter two sites, the surrounding connective tissue may show a tendency towards encapsulation of the lymphocystis cell.

Again, typical lymphocystis cells, measuring 281.2 x 395.2 microns, were encountered in the ovary (Pl. VIII, Fig. 17). These were closely surrounded by developing oöcytes. No fibrous responses to the presence of lymphocystis cells were noted in this region.

Cytological details of lymphocystis cells occupying the visceral organs

correspond closely with the fully developed cells of the cutaneous lesions. There is this difference, however, in that there occurs no massing of cells to form tumors. The cells appear as isolated units and in no instances were young, growing cells encountered in these viscera. No lymphocystis cells were found in the liver or kidney. In one of our diseased fish, the liver tissue showed evidence of hyperplasia of biliary ducts, but the cause for these changes at the present time remains uncertain.

Observations on Lymphocystis in the New York Aquarium.

Observations on lymphocystis disease among marine fishes in the New York Aquarium indicate that the disease appears at the height of the summer and has a tendency to disappear in late autumn and winter. Furthermore, outbreaks among fish showing the disease were invariably successive, *i.e.*, one fish may show the disease first, then another, etc., but not all simultaneously. Generally, there is a definite tendency for the infected fish to recover; the disease was seldom found to be fatal. Fish which showed the external manifestation of the disease have been kept for long periods (one year or more) after the external evidence had disappeared. Such fish seemed normal in all other respects.

It is interesting to point out that other species of fish kept in the same tank with infected specimens never showed signs of lymphocystiosis. Joseph (1918) reported similar findings. For example, such forms as *Mugil*, *Crenilabrus* and *Blennius* kept in the same tank with infected *Sargus*, the sunbream, showed no evidence of lymphocystis. In our observations, however, fishes kept in the same tank with lymphocystis infected specimens were usually more closely related forms and originally lived in the same region or habitat.

Attempts at Transmission.

Fifty killifish, *Fundulus heteroclitus*, were used for purposes of experimental transmission. A heavily infected orange filefish was used as the donor. The fishes were kept at 21° C. and in sea-water having a specific gravity of 1.0260. Ten of the fish were scarified and rubbed with lymphocystis material; ten were injected interperitoneally with an emulsion of infected material made up in pure sea-water; ten killifish were fed lymphocystis material; 20 fish were used as controls. The experimental fish were examined at the end of 1, 2, 3, and 4 weeks; 2, 3, and 4 months later. All results were negative. These findings, together with the observational data given above, may indicate that the disease is host-specific.

It is interesting to mention here, that in a recent contribution, Weissenberg (1939) also obtained negative results in attempting to transmit lymphocystis from infected pike-perch (*Stizostedion vitreum*) to both marine and fresh-water killifishes (*Fundulus heteroclitus* and *F. diaphanus*).

Discussion.

Evidence obtained from studies on lymphocystis disease occurring on the orange filefish from Sandy Hook Bay, N. J., is submitted showing that the lesions are not limited to cutaneous areas but that typical hypertrophied connective tissue cells occur also in the spleen, ovary and gastro-intestinal tract. Fully grown lymphocystis cells were found in these organs as solitary cells and cytologically correspond to similar cells in skin lesions. It will require the study of further material to determine the origin and fate of these enlarged cells involving the structures of the visceral organs. They may represent metastatic cells from cutaneous lesions which have found

their way to these visceral sites by way of vascular or lymphatic vessels, and have continued their development to the mature state in a new environment. Another explanation, of possibly greater significance, is that the agent causing the lymphocystiosis is ingested and gains access to the body and skin of the host through a portal of entry represented by the mucosa of the gastro-intestinal tract. It is interesting in this connection to point out that Weissenberg (1914, 1921) has described the transmission of lymphocystis disease by the feeding method in the European fishes, *Acerina cernua* and *Pleuronectes flesus*. Recently (still unpublished) he was able to transfer the lymphocystis by this method from diseased pike-perch of one locality to non-diseased individuals of the same species from a widely separated locality (see Weissenberg, 1939).

Weissenberg (1938) has presented data on lymphocystis disease in the orange filefish (*Aleutera schoepfii* = *Ceratacanthus schoepfii*) present in the Philadelphia Aquarium. He was able to trace the development of the connective tissue cells from those measuring 25-46 microns, and each containing a reticulated inclusion body on one side of the nucleus, to cells measuring 312-515 microns, containing inclusion bodies in the form of connecting networks arranged at the periphery and immediately below the thickened membrane. His interpretation concerning the development of the inclusions differs from the one given in the present paper. He states that during cell hypertrophy the inclusions appear "first as very thin points. Then they form small, compact, round bodies. Next they grow to oval discs with reticular or alveolar structure, and to fenestrated calottes. Finally, they spread out into the cytoplasm as fine networks." Weissenberg, however, does not indicate the origin of the original inclusion body, although he recognizes the fact that it is basochromatic in nature. The study of our material suggests that the system of cell inclusions present in the growing lymphocystis cell arises from one of two nuclear-like masses developed in early stages; and that therefore, such inclusions are perhaps of nuclear origin. A similar interpretation of the origin of inclusion bodies in this disease was first indicated by Woodcock (1904) and Awerinzew (1911), both reportng the inclusions as forming a "chromidial ring" derived from the nucleus.

Weissenberg (1914-1939) was first to consider lymphocystis as a cell hypertrophy of the host connective tissue, postulating that the causative agent is an intracellular virus probably liberated by the inclusion bodies. The same author was able to transmit the disease experimentally to normal fish by feeding infected tissue. Critical tests have not been made as yet to prove definitely that a virus is involved as a causative agent.

Summary.

1. Lymphocystis disease is described in orange filefish (*Ceratacanthus schoepfii*) from Sandy Hook Bay, New Jersey.

2. Histological studies reveal that this disease is not merely a cutaneous one, but that internal organs such as the gastro-intestinal tract, spleen and ovary are involved as well.

3. The present observations on progressive stages of cell hypertrophy support Weissenberg's interpretation that the disease results in a hypertrophy of connective tissue cells of the host.

4. The origin and the development of the cell inclusions from one of two nuclear masses formed in certain early stages of the disease is suggested.

5. Certain stages in the healing process of the disease are described.

6. Observations and attempts at experimental transmission indicate that lymphocystiosis may be species-specific.

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EXPLANATION OF PLATES.

Plate I.

Figs. 1 and 2. Cutaneous tumors caused by lymphocystis infection. These were taken from fins of orange filefish caught at Sandy Hook Bay, N. J.

Fig. 3. Typical nodule of lymphocystis tumor. 40 ×. Stained with Giemsa's. **(E)** epithelium; **(L)** lymphocystis cells; **(M)** meshwork of membrane material surrounding lymphocystis cells.

Plate II.

Fig. 4. Microscopic section through lymphocystis disease of the skin. 125 ×. H-E. **(N)** pedunculated nodule composed of fully developed cells, lying in a meshwork of cell membrane material. Note scanty connective tissue stroma containing lymphocytes. **(X)** area of growing lymphocystis cells in various stages of hypertrophy.

Fig. 5. Section as in Fig. 4, but at a different level. 125 ×. **(E)** cutaneous epithelium; **(X)** nest of growing cells; **(Y)** fully developed lymphocystis cells. Note the progressive stages of cell hypertrophy.

Plate III.

Fig. 6. Young lymphocystis cells. 250 ×. H-E. **(A)** "Binucleate" stage; **(B)** one nucleus appears compact; the other show signs of vacuolization.

Fig. 7. Young lymphocystis cells. 500 ×. H-E. Higher magnification of a section like that shown in Fig. 6, but at a different level. Note the two types of nuclear masses.

Plate IV.

Fig. 8. Young lymphocystis cells. 300 ×. H-E. Note the various "binucleate" stages.

Fig. 9. Lymphocystis cells slightly older than those shown in preceding photomicrographs. This figure shows the reticulate inclusions beginning to disperse.

Plate V.

Fig. 10. Fully matured lymphocystis cell, completely hypertrophied. 200 ×. H-E. **(N)** nucleus; **(NL)** nucleolus; **(I)** peripheral cytoplasmic inclusions; **(M)** cell membrane; **(XI)** peri-nuclear inclusion bodies.

Fig. 11. Section of cutaneous region showing healing stages from lymphocystis disease. 100 ×. H-E. **(H)** collapsed hyalin and greatly distorted lymphocystis cell membrances found in dense fibrous (scar) tissue **(S)**.

Plate VI.

Fig. 12. Completely hypertrophied lymphocystis cell in the spleen. 150 ×. H-E. As a result of fixation the cell has shrunken away from the concentrically arranged splenic tissue.

Fig. 13. Degenerated lymphocystis cell in the spleen. 150 ×. H-E. Note rupture of cell membrane between **(R)** and **(S)**, permitting invasion of splenic tissue **(Z)**.